

CLAIMS

What is claimed is:

- 5 1. A method of producing an antibody in the milk of a transgenic mammal, comprising:
providing a transgenic mammal whose somatic and germ cells comprise a
sequence encoding an exogenous heavy chain variable region or antigen binding
fragment thereof, at least one heavy chain constant region, or a fragment
thereof, and a hinge region, operably linked to a promoter which directs
10 expression in mammary epithelial cells, wherein said hinge region has been
altered from the hinge region normally associated with the heavy chain constant
region.
- 15 2. The method of claim 1, wherein at least 70% of the antibodies present in the milk are
in assembled form.
- 20 3. The method of claim 1, wherein said transgenic mammal further comprises a
sequence encoding a light chain variable region, or antigen binding fragment
thereof, and a light chain constant region or functional fragment thereof,
operably linked to a promoter which directs expression in mammary epithelial
cells.
- 25 4. The method of claim 1 further comprising the step of obtaining milk from said
transgenic mammal, to thereby provide an antibody composition.
5. The method of claim 4 further comprising the step of purifying the exogenous
antibody from the milk produced by said transgenic mammal.

6. The method of claim 1 wherein said promoter is a promoter selected from the group consisting of: casein promoter, lactalbumin promoter, beta lactoglobulin promoter and whey acid protein promoter.
- 5 7. The method of claim 1 wherein said transgenic mammal is a mammal selected from the group consisting of: cow, goat, mouse rat, sheep, pig and rabbit.
8. The method of claim 1 wherein the antibody is an antibody selected from the group consisting of: IgA, IgD, IgM, IgE or IgG.
- 10 9. The method of claim 1 wherein the antibody is an IgG antibody.
10. The method of claim 1 wherein the antibody is an IgG4 antibody.
- 15 11. The method of claim 10 wherein all or a portion of the hinge region of said antibody has been altered.
12. The method of claim 10, wherein all or a portion of the hinge region of the antibody has been replaced, e.g. replaced with a hinge region or portion thereof which
20 differs from the hinge region normally associated with said heavy chain constant region.
13. The method of claim 10, wherein the amino acid sequence of the hinge region of the antibody differs from the amino acid sequence of the hinge region naturally
25 associated with said heavy chain constant region by at least one amino acid residue.
14. The method of claim 1, wherein at least one of the nucleic acid residues of the nucleic acid sequence encoding the hinge region of the antibody differs from the

naturally occurring nucleic acid sequence of the hinge region naturally associated with said heavy chain constant region.

15. The method of claim 12, wherein the hinge region of the antibody, or portion thereof, has been replaced with the hinge region, or portion thereof, of an antibody other than an IgG4 antibody.
16. The method of claim 12 wherein the hinge region, or portion thereof, of the antibody has been replaced with a hinge region, or portion thereof, derived from an antibody selected from a group consisting of: IgG1, IgG2 and IgG3.
17. The method of claim 12 wherein the hinge region of the antibody, or a portion thereof, has been replaced with a hinge region, or portion thereof, derived from an antibody selected from a group consisting of: IgA, IgD, IgM and IgE.
18. The method of claim 12 wherein one or more amino acids of the hinge region have been replaced with an amino acid corresponding to that position in an antibody other than an IgG4 antibody.
19. The method of claim 15 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgA, IgD, IgM and IgE.
20. The method of claim 15 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgG1, IgG2 and IgG3.
21. The method of claim 10, wherein a serine residue of the hinge region has been replaced with a proline residue.

22. The method of claim 10, wherein a serine residue at amino acid number 241 of the hinge region has been replaced with a proline residue.
23. The method of claim 10, wherein at least one amino acid in the hinge region other
5 than a cysteine residue is replaced with a cysteine residue.
24. The method of claim 10 wherein at least 1 glycosylation site of the antibody is altered.
- 10 25. The method of claim 24, wherein at least one glycosylation site in the heavy chain or light chain is altered.
26. The method of claim 24, wherein at least one glycosylation site in the hinge region of the heavy chain is modified.
- 15 27. The method of claim 1 wherein the antibody is humanized.
28. The method of claim 1 wherein the antibody is chimeric.
- 20 29. The method of claim 1 wherein the antibody is a human antibody.
30. The method of claim 1 wherein the milk of the transgenic mammal is essentially free from a half molecule form of the exogenous antibody.
- 25 31. The method of claim 1 wherein the ratio of assembled exogenous antibody to half forms of the antibody present in the milk of a transgenic mammal are at least 2:1, 3:1, 4:1 or 5:1.

32. A method of producing a transgenic mammal whose somatic and germ cells comprise a modified antibody coding sequence wherein said modified antibody coding sequence encodes an antibody molecule or portion thereof expressible in milk, comprising a modified hinge region, said method comprising the steps of:
- 5 introducing into a mammal a construct comprising a sequence encoding an exogenous heavy chain variable region or antigen binding fragment thereof, at least one heavy chain constant region or a fragment thereof, and a hinge region, operably linked to a promoter which directs expression in mammary epithelial cells, wherein said hinge region has been altered from the hinge region normally
- 10 associated with the heavy chain constant region.
33. The method of claim 33, wherein said hinge region has been altered such that at least 70% of the exogenous antibodies present in the milk of the transgenic mammal are in assembled form.
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34. The method of claim 33, wherein said modified antibody coding sequence further comprises a sequence encoding a light chain variable region or antigen binding fragment thereof and a light chain constant region or functional fragment thereof, operably linked to a promoter which directs expression in mammary
- 20 epithelial cells.
35. The method of claim 33 wherein the promoter is a promoter selected from the group consisting of: casein promoter, lactalbumin promoter, beta lactoglobulin promoter and whey acid protein promoter.
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36. The method of claim 33 wherein the transgenic mammal is a mammal selected from the group consisting of: cow, goat, mouse rat, sheep, pig and rabbit.
37. The method of claim 33 wherein the antibody is an antibody selected from the
- 30 group consisting of: IgA, IgD, IgM, IgE or IgG.

38. The method of claim 33 wherein the antibody is an IgG antibody.

39. The method of claim 33 wherein the antibody is an IgG4 antibody.

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40. The method of claim 40 wherein all or a portion of the hinge region of the antibody has been altered.

41. The method of claim 40 wherein all or a portion of the hinge region of the antibody has been replaced, e.g. replaced with a hinge region or portion thereof which differs from the hinge region normally associated with said heavy chain variable region or said constant region.

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42. The method of claim 40, wherein the amino acid sequence of the hinge region of the antibody differs from the amino acid sequence of the hinge region naturally associated with said heavy chain constant region by at least one amino acid residue.

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43. The method of claim 33, wherein at least one of the nucleic acid residues of the nucleic acid sequence encoding the hinge region of the antibody differs from the nucleic acid sequence of the hinge region naturally associated with said heavy chain constant region.

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44. The method of claim 44, wherein the hinge region of the antibody, or portion thereof, has been replaced with the hinge region, or portion thereof, of an antibody other than an IgG4 antibody.

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45. The method of claim 42 wherein the hinge region, or portion thereof, of the antibody has been replaced with a hinge region, or portion thereof, derived from an antibody selected from a group consisting of: IgG1, IgG2 and IgG3.
- 5 46. The method of claim 42 wherein the hinge region of the antibody, or a portion thereof, has been replaced with a hinge region, or portion thereof, derived from an antibody selected from a group consisting of: IgA, IgD, IgM and IgE.
- 10 47. The method of claim 42 wherein one or more amino acids of the hinge region have been replaced with an amino acid corresponding to that position in an antibody other than an IgG4 antibody.
- 15 48. The method of claim 48 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgA, IgD, IgM and IgE.
49. The method of claim 48 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgG1, IgG2 and IgG3.
- 20 50. The method of claim 40, wherein a serine residue of the hinge region has been replaced with a proline residue.
51. The method of claim 40, wherein a serine residue at amino acid number 241 of the hinge region has been replaced with a proline residue.
- 25 52. The method of claim 40, wherein at least one amino acid in the hinge region other than a cysteine residue is replaced with a cysteine residue.
53. The method of claim 40 wherein at least one glycosylation site of the antibody is altered.

54. The method of claim 54 wherein at least one glycosylation site in the heavy chain or light chain is altered.
- 5 55. The method of claim 40, wherein at least one glycosylation site in the hinge region of the heavy chain is modified.
56. The method of claim 33 wherein the antibody is humanized.
- 10 57. The method of claim 33 wherein the antibody is a human antibody.
58. The method of claim 33 wherein the antibody is chimeric.
- 15 59. The method of claim 33, wherein said hinge region has been altered such that the milk of the transgenic mammal is essentially free from a half molecule form of the exogenous antibody.
60. The method of claim 33 wherein the ratio of assembled exogenous antibody to half forms of the antibody present in the milk of a transgenic mammal are at least 2:1, 3:1, 4:1 or 5:1.
- 20 61. The method of claim 60 wherein the antibody is an antibody selected from the group consisting of: IgA, IgD, IgM, IgE or IgG
- 25 62. A method of producing a transgenic mammal capable of expressing an assembled exogenous antibody or portion thereof in its milk, the method comprising:
introducing into a mammal a construct comprising a sequence encoding a light chain of exogenous antibody operably linked to a promoter which directs expression in mammary epithelial cells; and

introducing into the mammal a construct comprising a sequence encoding a mutagenized heavy chain of the exogenous antibody or a portion thereof operably linked to a promoter which directs expression in mammary epithelial cells, wherein the heavy chain or portion thereof comprises a hinge region which has been altered such that at least 70% of the exogenous antibodies present in the milk are in assembled form.

63. A method of producing a transgenic mammal capable of expressing an assembled exogenous antibody in its milk, the method comprising:

providing a cell from a transgenic mammal whose germ and somatic cells comprise a sequence encoding a light chain of an exogenous antibody operably linked to a promoter which directs expression in mammary epithelial cells; and introducing into the cell a construct comprising a sequence encoding a mutagenized heavy chain of the exogenous antibody or a portion thereof operably linked to a promoter which directs expression in mammary epithelial cells, wherein the heavy chain, or portion thereof comprises a hinge region which has been altered such that at least 70% of the exogenous antibodies present in the milk are in assembled form.

64. A composition comprising a milk component and an antibody component, wherein said antibody component comprises an exogenous antibody, or fragment thereof, having a hinge region, wherein said hinge region has been altered from the hinge region normally associated with the antibody.

65. The composition of claim 63, wherein at least 70% of the exogenous antibodies present in said composition are in assembled form.

66. The composition of claim 63, wherein said hinge region has been altered such that at least 70% of the exogenous antibodies present in said composition in assembled form.

67. The composition of claim 63 wherein the antibody is an antibody selected from the group consisting of: IgA, IgD, IgM, IgE or IgG.
- 5 68. The composition of claim 63 wherein the antibody is an IgG antibody.
69. The composition of claim 67 wherein the antibody is an IgG4 antibody.
70. The composition of claim 63 wherein all or a portion of the hinge region of the
10 antibody has been altered.
71. The composition of claim 63, wherein all or a portion of the hinge region of the antibody has been replaced, e.g. replaced with a hinge region or portion thereof which differs from the naturally occurring hinge region normally associated
15 with the antibody.
72. The composition of claim 63, wherein the amino acid sequence of the hinge region of the antibody differs from the amino acid sequence of the hinge region of the naturally occurring antibody by at least one amino acid residue.
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73. The composition of claim 63, wherein the hinge region of the antibody, or portion thereof, has been replaced with the hinge region, or portion thereof, of an antibody other than an IgG4 antibody.
- 25 74. The composition of claim 72 wherein the hinge region, or portion thereof, of the antibody has been replaced with a hinge region, or portion thereof, from an antibody selected from a group consisting of: IgG1, IgG2 and IgG3.

75. The composition of claim 72 wherein the hinge region of the antibody, or a portion thereof, has been replaced with a hinge region, or portion thereof, derived from an antibody selected from a group consisting of: IgA, IgD, IgM and IgE.
- 5 76. The composition of claim 63 wherein one or more amino acids of the hinge region have been replaced with an amino acid corresponding to that position in an antibody other than an IgG4 antibody.
- 10 77. The composition of claim 75 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgA, IgD, IgM and IgE.
78. The composition of claim 75 wherein the antibody other than an IgG4 antibody is an antibody selected from the group consisting of: IgG1, IgG2 and IgG3.
- 15 79. The composition of claim 63, wherein a serine residue of the hinge region has been replaced with a proline residue.
80. The composition of claim 63, wherein a serine residue at amino acid number 241 of the hinge region has been replaced with a proline residue.
- 20 81. The composition of claim 63, wherein at least one amino acid in the hinge region other than a cysteine residue is replaced with a cysteine residue.
82. The composition of claim 63 wherein at least one glycosylation site of the antibody is altered.
- 25 83. The composition of claim 63, wherein at least one glycosylation site in the heavy chain or light chain of the antibody is altered.

84. The composition of claim 82, wherein at least one glycosylation site in the hinge region of the heavy chain of the antibody is modified.

85. The composition of claim 63 wherein the antibody is humanized.

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86. The composition of claim 63 wherein the antibody is a human antibody.

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87. The composition of claim 63, wherein said hinge region has been altered such that the composition is essentially free from a half molecule form of the exogenous antibody.

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88. The composition of claim 63 wherein the ratio of assembled exogenous antibody to half forms of the antibody present in the composition is at least 2:1, 3:1, 4:1 or 5:1.

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89. A nucleic acid comprising a sequence encoding a heavy chain variable region and a heavy chain constant region, operably linked to a promoter which directs expression in mammary epithelial cells, wherein the heavy chain or portion thereof comprises a hinge region which has been altered such that at least 70% of the exogenous antibodies present in milk are in assembled form.